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Behavioral and Pharmacological Responses Following Acrylamide Exposure in Rats

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16. ABSTRACT

The onset of acrylamide related toxicity and indications of recovery were evaluated using several behavioral tasks. Rats were chronically housed and tested for the first 15 weeks of the study in running wheels where food and water consumption and activity were continously monitored. Seperate groups of animals were repeatedly tested on at least one additional behavioral task: and operant schedule FR20 GO/NO-GO, spontaneous and drug-induced locomotor activity, drug-induced asymmetric rotation, or splaying of the hindlimbs. Animals were administered acrylamide monomer in their drinking water (loo ppm) for a six week period. Behavioral evaluations were initiated up to six weeks prior to acrylamide exposure and continued up to 12 weeks after termination of the exposure. Operant behavior and spontaneous locomotor activity in an open field were not affected by acrylamide exposure. Nocturnal activity in running wheels was decreased, and hindlimb splay increased by exposure to acrylamide. Subsequent to injections of d-amphetamine locomotor activity in an "open field" was markedly increased following exposure to acrylamide. d-Amphetamine induced asymmetric rotation was also altered following acrylamide exposure. It is suggested that this altered pharmacological response was due to an effect of acrylamide on hepatic mechanisms or on central dopaminergic function.

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Behavioral and Pharmacological Responses Following Acrylamide Exposure in Rats[1]

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Acrylamide Hindlimb splay d-Amphetamine Running wheel activity Locomotor activity
Operant schedule Rats

LABORATORY animals chronically exposed to acrylamide gradually develop a bilateral hindlimb weakness, unsteady gait, and if the exposure continues, paresis [10]. These effects are reversible following the removal of the neurotoxin. However, clinical recovery is incomplete in severely intoxicated animals who continue to exhibit residual spasticity and ataxia [23,24]. Other studies suggest that, while clinical signs of peripheral neuronal damage may predominate, there are morphological alterations evident for nervous tissue within the central nervous system as well [21]. In addition, it has been found that acrylamide exposure can cause depletion of several neurotransmitters in the CNS [1,8] as well as affect amino acid transport mechanisms [5]. As a whole, these findings suggest that acrylamide may compromise a number of behaviors which require intact CNS function. The purpose of the present study was to evaluate the effects of acrylamide exposure on (a) consummatory behavior, (b) nocturnal activity in the home environment, (c) spontaneous diurnal activity in a novel environment, and (d) performance on an operant task. Splay, a measure of hindlimb function, was also included in this testing protocol. Evidence relating acrylamide exposure to central dopaminergic pathways [1] suggested that two other behaviors, locomotor activity and rotation, be evaluated before and after challanges with d-amphetamine.

METHOD

Animals and Housing

Forty eight male Long-Evans hooded rats of approximately 24 days of age were obtained from Charles River Laboratories (Willmington, MA). Animals were housed in groups of three in hanging stainless steel cages for three weeks.

At approximately 45 days of age animals were transferred to Wahman® stainless steel running wheels. Attached to each wheel was a rectangular home cage measuring 25×15×12.5 cm with a sliding door between the home cage and the wheel. Food (Powdered chow #5001; Ralston Purina Co., St. Louis, MO) and tap water were generally available in the housing unit. Exceptions to this ad lib feeding schedule are noted below. Animals were weighed and food and water (or solution) measured and replenished on Mondays and Fridays. Corn cob cellulose bedding (Anderson Cob Division, Maumee, OH) was changed weekly on Fridays. The replenishment of food and water and bedding changes were

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always deferred until the animals to be tested in the behavioral tasks for that day had been removed from their residential wheels. The light/dark cycle had a ratio of 12:12 with the light cycle commencing at 0700 hours. Ambient room temperature remained constant at 23±1°C. After 15 weeks residence in the Wahman® Chambers animals were transferred to stainless steel hanging cages where they were individually housed under the same temperature and lighting conditions.

Task Assignment

The 48 animals were randomly subdivided into five groups. Three of the five groups were each assigned to a particular task for which they were to be assessed throughout the experiment. For convenience, each group was given the name of their respective task: locomotor activity (Group ACT; n=10), operant performance (Group: OPER; n=10), and rotation (Group: ROT; n=8). Each group was periodically removed from the running wheels and evaluated in their respective task. The remaining two groups were maintained in the running wheels and side enclosures continuously for 15 weeks (except for those occasions when animals were weighed). These latter two groups were distinguished from one another by their exposure history; one group never receiving acrylamide and the other receiving acrylamide on the same schedule as that of the former three groups (see section on exposure protocol). The non-acrylamide exposed group of animals was primarily a negative control group (Group: NCTL) for the running wheel task. Those animals exposed to acrylamide but not tested for other behaviors while housed in the running wheels (Group: RWCTL) served primarily as a control group for determining the impact of the three behavioral tasks (locomotor activity, operant behavior, and rotation) on the running wheel task.

Exposure Protocol

Acrylamide solutions (100 ppm) were prepared from A.C.S. chemical grade acrylamide monomer (No. 8887: Sigma Chemical Co., St. Louis, MO) dissolved in distilled water. At this concentration the cumulative dose for an average animal over a six week period approaches 600 mg/kg. This dose is within a range where hindlimb weakness has been observed [10,19].

All groups except NCTL had their drinking water replaced by acrylamide solutions after six weeks residence in the running wheels. Tap water again replaced acrylamide solutions for the four groups of exposed animals after a six week period of exposure (week 13 of the study). All animals were maintained on tap water for the remainder of the study.

General Testing Protocol

The overall testing protocol for the present study is summarized in Table 1. In addition to the between group controls mentioned earlier, baseline data was collected for each task, prior to acrylamide exposure. This design maximized the use of each animal serving as its own control. The study was intended to last 15 weeks, six weeks for baseline data collection, six weeks for testing during exposure to acrylamide, and three weeks for post-exposure testing. Animals were tested for six-weeks prior to exposure, however equipment failures and methodological difficulties reduced the usable amount of data collected during the baseline period to four weeks for rotation and to one week for both locomotor activity and operant behavior. The full six week baseline period was realized for the running wheel task. The post-exposure period was extended up to 12 weeks beyond the exposure period because of persistent changes observed on some tasks.

Behavioral Assessment

Tests of activity in running wheels. Activity in a familiar home environment was assessed by recording the number of turns animals made in running wheels. (Wahman® Model #LC-34) during the nocturnal portion of their light/dark cycle; a 12 hr period in which all five groups of animals were left undisturbed. Animals were locked out of the running wheels from 0800 until 1700 hours on Mondays, Wednesdays, and Fridays throughout the study. This restriction was necessary to equate the accessibility to running wheels across all animals in all groups. When access to the wheels was not denied the activity of rats during this time period was less than 10% of their total 24 hour activity.

Splayed leg tests. Several different measures have been used to determine peripheral neuromuscular dysfunction in the hindlimbs of experimental animals. These include measures of gait parameters [18], the latency to remain on an inclined plane [9], the use of a strain gauge [3], and a measure of the distance (splay) between the hindlimbs of an animal dropped from a fixed height [7]. In the absence of any data comparing these methods, we choose to employ hindlimb splay primarily because of its ease of implementation. Briefly, this procedure required that animals be dropped from a fixed height (30.5 cm) onto a clear sheet of paper after inking the feet with diluted food dye. This protocol was repeated four times. The mean distance between the fourth digits of the two hindfeet served as the measure of splay. Prior accounts of peripheral neuropathies produced by acrylamide in the drinking water or diet of rats indicate that hindlimb deficits are not evident until a cumulative dose of at least 367 mg/kg is attained [10,19]. Consequently, we initiated this procedure during the third week of exposure when animals had a mean cumulative dose of acrylamide of 272 mg/kg and continued it through the fourth week of recovery. Tests were made on NCTL and RWCTL animals. These two groups were tested once each week at a time during the day when animals were routinely weighed.

Locomotor activity tests with and without drug challenge. Spontaneous activity in a more novel environment then the home cage was assessed for the ACT group. These animals were also tested for d-amphetamine induced locomotor activity. Testing occurred three days a week. Animals in all five groups were locked out of their running wheels and weighed; and the ACT group transferred to transparent cages measuring 29×34×45 cm at 0900 hours on Mondays, Wednesdays, and Fridays.

Two red fluorescent lights with peak emission spectra of 630 nm (range: 575–725 nm) were positioned above the cages. Each cage rested on a platform containing an array of seven photocells with an average interphotocell distance of 13 cm. The photocell output was interfaced to a microcomputer which monitored the activity of each animal. Data was accumulated and stored in five minute blocks on magnetic tape.

After monitoring spontaneous activity for one hour, animals received either a 1.0 mg/kg SC injection of d-amphetamine sulfate or its vehicle, bacteriostatic saline. Activity was subsequently recorded for an additional two

TABLE 1

BEHAVIORAL TESTING PROTOCOL*

Week	Amphetamine and Task Control (Group:RWCTL)	Negative Control (Group:NCTL)	Locomotor Activity (Group:ACT)	Rotation (Group:ROT)	Operant (Group:OPER)
Baseline Period					
1	rw	rw	rw act†	rw rot†	rw oper†
2	rw	rw	rw act†	rw rot†	rw oper†
3	rw	rw	rw act†	rw rot	rw oper†
4	rw	rw	rw act†	rw rot	rw oper†
5	rw	rw	rw act†	rw rot	rw oper†
6	rw	rw	rw act	rw rot	rw oper
Exposure Period					
7	rw	rw	rw act	rw rot	rw oper
8	rw	rw	rw act	rw rot	rw oper
9	rw sl	rw sl	rw act	rw rot	rw oper
10	rw sl	rw sl	rw act	rw rot	rw oper
11	rw sl	rw sl	rw act	rw rot	rw oper
12	rw sl	rw sl	rw act	rw rot	rw oper
Postexposure Period					
13	rw sl	rw sl	rw act	rw rot	rw oper
14	rw sl	rw sl	rw act	rw rot	rw oper
15	rw sl	rw sl	rw act	rw rot	rw oper
16	sl	sl	act		
17	act	act	act		
18	act	act	act		
19			act		
20					
21	rot	rot		rot	
22	rot	rot			
23			act		
24	act	act			

*Abbreviations: rw= running wheel; sl=splay leg test; act=locomotor activity, both spontaneous and drug-induced; rot=rotation, both spontaneous and drug-induced; oper=operant performance.

†No data for these sessions.

hours. d-Amphetamine was administered on Mondays and Fridays and saline was given each Wednesday.

Groups NCTL and RWCTL were also tested for spontaneous and drug-induced locomotor activity during the 17th, 18th, and 24th week of the study.

Rotation. Rotation in non-lesioned rats is thought to reflect an intrinsic asymmetry in striatal dopamine which is accentuated by d-amphetamine [12, 15–17]. This behavior, like that of locomotor activity, can therefore be used to provide one index of dopaminergic function and of the pharmacological responsiveness of an animal to d-amphetamine. Rotation was assessed every Monday and Friday for the ROT group. Animal rotation was recorded by attaching one end of a wire to a harness around the animal and the other end to a vane which interrupted four photocells located 90 degrees from one another. Once the harness was positioned the animal was placed below the vane in a cylindrical chamber (25 cm dia.). A microcomputer was programmed to determine the number of 360 degree rotations an animal made, both clockwise and counterclockwise. This information was accumulated simultaneously for eight experimental chambers, every 15 minutes for a 3 hour session. Spontaneous rotation was recorded for one-half hour and d-amphetamine induced rotation (1.0 mg/kg as salt SC) was recorded for an additional two hours.

Operant behavior. Performance on an operant schedule requires intact mechanisms of motivation, coordination of voluntary movement, the perception of controlling stimuli, and recognition of some relationship between a stimulus and contingencies of reinforcement. Disruptions of this behavior can therefore reflect functional damage to neuronal cell populations which collectively maintain these adaptive functions. Animals in group OPER were trained to lever press for a liquid reinforcement (1 part Carnation® Evaporated Milk, 2 parts water) in Colbourne operant chambers. Operant animals were maintained at between 70–85% of the body weight of RWCTL animals. Body weight was maintained by a restricted feeding schedule. The absolute duration of food availability varied from 60–75 minutes on those days which preceded operant testing to 3–4 hours on days which preceded no test days. No food was available for a minimum of 12 hours prior to testing an operant animal.

Operant animals were initially trained to lever press on a fixed ratio 1 (FR1) schedule of reinforcement. The schedule was then gradually increased to FR20 while, simultaneously, a GO/NO-GO light discrimination was super-imposed. The

TABLE 2

MEAN WEEKLY DOSES OF ACRYLAMIDE (mg/kg) DURING THE SIX WEEK PERIOD OF EXPOSURE*

Group:		Running Wheel (RWCTL) (n=10)		Locomotor Activity (ACT) (n=10)		Rotation (ROT) (n=8)		Operant (OPER) (n=10)	
Week of Exposure	Week of Study	Weekly Dose	Cumulative Dose	Weekly Dose	Cumulative Dose	Weekly Dose	Cumulative Dose	Weekly Dose	Cumulative Dose
1	7	90.8	90.8	90.2	90.2	93.6	93.6	99.5	99.5
2	8	91.6	182.4	94.1	184.3	104.9	198.5	107.9	207.4
3	9	89.6	272.0	100.4	284.7	95.8	294.3	94.2	301.6
4	10	93.4	365.4	87.8	372.5	88.0	382.3	101.5	403.1
5	11	86.6	452.0	86.0	458.5	87.8	470.0	82.4	485.5
6	12	73.2	525.2	77.7	536.2	76.9	546.9	81.8	567.3

*No significant differences in dose ($p>0.10$) occurred during any week of exposure.

GO period (light-on period during which reinforcement could be obtained) was fixed at one minute. The NO-GO period varied, first on a variable interval 20 second (VI20) schedule and, after three weeks, on a VI30 schedule in order to decrease the number of responses emitted during the NO-GO periods. This latter schedule remained in effect until the termination of the experiment.

Statistical Analyses

Proportionalities between group means and variance estimates required that all activity data be square-root transformed prior to parametric statistical analyses [25]. This included all locomotor activity measures, activity in running wheels, and rotational behavior. Post-hoc comparisons were made using the Neuman-Keuls test with experiment-wise error set at $p<0.05$.

RESULTS

Fluid and Food Consumption and Body Weight

Analyses of fluid and food consumption and body weight excluded the data of OPER animals because of the necessity to restrict the dietary intake of these animals. For the remaining groups of animals these measures were subjected to analyses of variance (ANOVA). Separate ANOVAs were computed for each measure at each of the three periods: baseline (6 weeks), exposure (6 weeks) and post-exposure (3 weeks).

Each was a two-factor ANOVA with group (NCTL, RWCTL, ACT ROT) as a between subject source of variance and week as a repeated measure. In general, fluid consumption, food intake, and body weight did not differ between any of the groups during any period of the experiment. The only exception to this was a tendency for ROT animals to eat slightly less (11%) than the other three groups during the second week of exposure and for NCTL animals to eat slightly more (9.7%) than the other three groups during the fifth week of exposure, $F(15,170)=1.88$, $p<0.05$; and post-hoc comparisons, $p<0.05$.

Acrylamide Dosage

The lack of significant differences between the groups in fluid intake or in weight suggested that exposed animals received equivalent external doses of acrylamide. Table 2 and a two-factor ANOVA computed on the amount of acrylamide ingested per group over the six week period of exposure confirmed this equivalence, $F(3,34)<$, $p>0.10$; $F(15,170)<1$, $p>0.10$. It is notable that animals in the OPER group, who were maintained at a body weight 70–85% of that of RWCTL animals, ingested amounts of acrylamide per gram body weight which were equivalent to that of the other three groups of exposed animals.

Running Wheel Activity

The number of turns made per nocturnal period was averaged for each week. Figure 1 portrays the means of these averages for four groups of animals. Separate ANOVAs were computed for each of the three periods: baseline, exposure, and post-exposure. Each ANOVA factored group (NCTL, RWCTL, ROT, ACT) and week; where week was a repeated measure.

OPER animals showed marked decreases in nocturnal running wheel activity prior to acrylamide exposure. In all likelihood this was due to food deprivation. Consequently this group was excluded from all analyses of running wheel activity.

The activity of the remaining four groups increased over the baseline period, $F(5,170)=37.70$, $p<0.001$. Those animals who were evaluated for rotation had a tendency to exhibit less nocturnal activity in the running wheels. However, none of the four groups differed statistically from one another during the baseline period, $F(3,34)<1$, $p>0.10$.

During the six week period of exposure all groups showed a decline in their running wheel activity, $F(5,165)=38.31$, $p<0.001$. A statistically significant interaction between time and treatment condition, $F(15,165)=1.97$, $p<0.02$, indicated that the rate of decline for the four groups was not equivalent. Post-hoc comparisons indicated that the three groups of animals exposed to acrylamide (RWCTL, ACT, ROT) were less active than controls (NCTL) within three weeks after the onset of exposure (week 9 of the study) and that this difference persisted through the sixth week of exposure (week 12 of the study).

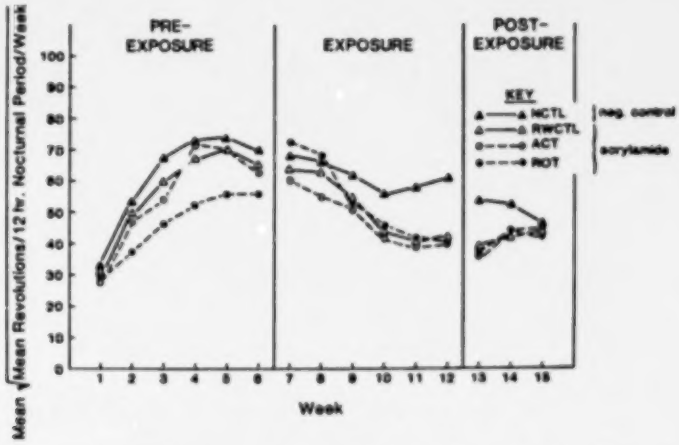

FIG. 1. Nocturnal activity in running wheels for four groups of animals chronically housed for 15 weeks. Statistically significant decreases occurred by the third week of exposure (cumulative dose ≈ 288 mg/kg).

During the three week post-exposure period differences between the activity of NCTL animals and animals previously exposed to acrylamide (ACT, ROT and RWCTL groups) decreased. An interaction between treatment and time, $F(6,64)=2.85$, $p<0.02$, and post-hoc comparisons, indicated that differences in activity between the non-exposed group (NCTL) and the three groups of animals previously exposed to acrylamide were no longer statistically significant by the third week after exposure was terminated (week 15 of the study). It is not clear whether these findings reflect recovery in acrylamide exposed animals or the often observed tendency of control animals to reduce their running wheel activity as a function of age.

Splay Leg Test

The distance between the fourth digit of each hindlimb was averaged for four consecutive "drops" per animal for each weekly test. The means of these scores served as the raw data for Fig. 2. Separate two factor ANOVAs were computed, one for the exposure period and one for the post-exposure period. Although a strong trend was evident for splay to increase in RWCTL animals during the period of exposure, this increase was not statistically significant, $F(1,18)=1.74$, $p>0.10$. However, the ANOVA on post-exposure splay revealed a significant treatment by week interaction, $F(3,54)=6.15$, $p<0.01$. Post-hoc comparisons indicated that splay for the RWCTL group was significantly increased over that of the NCTL animals for weeks 13 and 14 of the study. These differences abated by the 15th week (3rd week post-exposure).

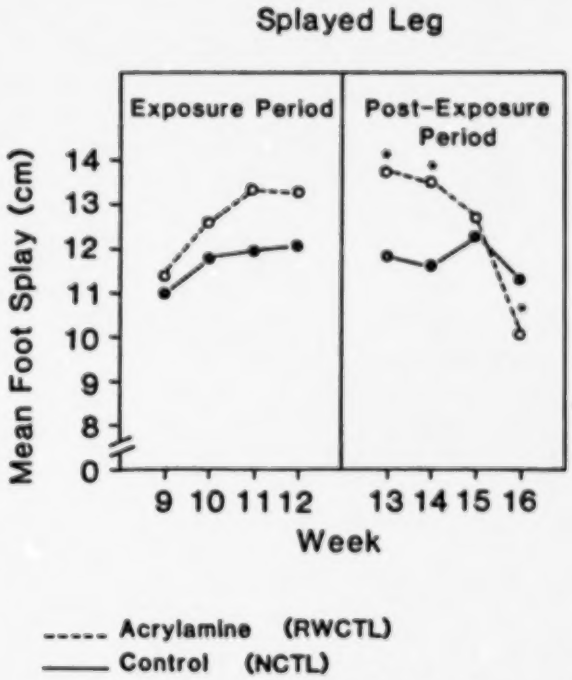

FIG. 2. Mean distance between the fourth digit of each hindlimb for RWCTL and NCTL groups (*$p<0.05$).

Locomotor Activity With and Without Drug Challenge

Locomotor activity for group ACT over an 18 week period is depicted in Fig. 3. Locomotor activity from only one of two sessions per week is recorded (Friday sessions). The graph presents mean spontaneous locomotor activity occurring during the one hour interval prior to d-amphetamine injections as well as locomotor activity occurring during the first and second hour after injection. Separate analyses were computed for spontaneous locomotor activity and for locomotor activity occurring one and two hours post-drug. Each of these were repeat measure ANOVAs. Separate repeat measure ANOVAs were performed on locomotor activity during the period of exposure (week 7–12) and on locomotor activity during the post-

TABLE 3
SUMMARY OF MAJOR STATISTICAL RESULTS FOR ACT GROUP

	During Exposure (Weeks 7–12)		After Termination of Exposure (Weeks 13–23)	
Locomotor Activity	F-value	Post-hoc comparisons*	F-value	Post-hoc comparisons*
Spontaneous	$F(6,54)=1.07$ N.S.	—	$F(5,54)=4.14$ $p<0.01$	week 23 ↑
First Hour Post-Drug	$F(6,54)=9.61$ $p<0.001$	week 12 ↑	$F(6,54)=6.54$ $p<0.001$	weeks 13–15 ↑
Second Hour Post-Drug	$F(6,54)=15.85$ $p<0.001$	weeks 9–12 ↑	$F(6,54)=21.33$ $p<0.001$	weeks 13–20 ↑

*Comparison to activity during sixth week baseline period (Neuman-Keuls test, $p<0.05$).

exposure period (weeks 13–23). Each of these analyses also included baseline locomotor activity prior to the introduction of acrylamide (week 6). The results of these analyses are summarized in Table 3.

Spontaneous locomotor activity did not change from baseline levels during the period of exposure. For the most part, spontaneous locomotor activity was also comparable to baseline levels after acrylamide exposure had been terminated. The only exception to this was a modest increase in spontaneous locomotor activity during the last week of testing (week 23).

The locomotor activity of ACT animals during the first hour after d-amphetamine injections increased over the six week exposure period. However, only locomotor activity during the sixth week of exposure (week 12 of study) was significantly greater than baseline levels (week 6 of study).

Locomotor activity during the second hour after d-amphetamine injections also increased during the six week exposure period. These increases were statistically significant by the third week of exposure to acrylamide (week 9 of the study).

Locomotor activity for both the first and second hour after drug challenge decreased following the removal of acrylamide from drinking solutions. For the first hour post-injection, locomotor activity levels returned to baseline within four to seven weeks after termination of exposure (weeks 16–19 of the study). For the second hour of drug-induced locomotor activity, a return to baseline locomotor activity levels occurred between 9 and 11 weeks after termination of exposure (weeks 21–23).

Long-term administration of d-amphetamine has been reported to progressively augment the locomotor stimulating effects of this drug ("reverse tolerance," [22]). While the present study used doses of d-amphetamine less than those reported to produce this effect (2 mg/kg/wk vs 7 mg/kg/wk), it was recognized that this phenomenon could be influencing the activity of animals in the current study; obscuring the extent to which acrylamide exposure, per se, was altering drug responsiveness. To dissassociate a possible "reverse tolerance" effect from a true effect of acrylamide on the locomotor stimulating effects of d-amphetamine, we employed a between groups design using negative control animals (Group: NCTL) and animals previously exposed to acrylamide (Group: RWCTL); neither of which had any prior experience with d-amphetamine or with the locomotor activity test situation.

The results of a drug challenge during weeks 17 and 18 of the study (1.0 mg/kg SC d-amphetamine sulfate) for these two groups are depicted in Fig. 4, along with data from group ACT who were repetitively administered d-amphetamine. A two-factor ANOVA with repeat measures over the 24, 5-minute intervals post-injection demonstrated that RWCTL animals were more active following d-amphetamine than were NCTL control animals, $F(1,18)=5.28$, $p<0.05$. It is also apparent from the figure that ACT animals were more active then either RWCTL or NCTL rats during this post-exposure period (week 17–18 of the study).

By week 24 of the study (12 weeks after termination of exposure) the augmented drug-response of the RWCTL and ACT groups was not evident, and the activity of all three groups (RWCTL, ACT, NCTL) were comparable, $F(2,25)<1$, $p>0.10$; $F(46,575)$, 1, $p>0.10$.

Rotational Behavior

The number of 360° turns emitted prior to drug injections was negligible for all sessions. Following d-amphetamine, rats turned in either a predominantly clockwise or counterclockwise direction. Each animal's predominent direction of turning was consistent both within a session and across sessions. In addition, turns in the non-dominant direction occurred only infrequently following drug administration. Consequently, data analysis focused upon turns made in the dominant direction of rotation which occurred subsequent to the administration of d-amphetamine.

The time-course of drug-induced rotation prior to (week 6 of study), during (week 12 of study), and following acrylamide exposure (week 15 of study) are shown in Fig. 5. It can be seen that the early peak responses were attenuated and the later responses augmented during and following exposure to acrylamide. These data suggested that the distribution of turns emitted following a drug-challenge was modified after exposure to acrylamide. To further characterize this change, summary values were computed for the 45 minute interval of each session where maximal rotations typically occurred (intervals 2, 3 and 4) and for the 45 minute interval during which drug-induced rotations typically began to wane (intervals 6, 7 and 8). These summary values (see Fig. 6) were then subjected to a two-factor ANOVA with repeated measures over the period-within-a-session (early period of maximal responding and late period of minimal responding) and the week of testing (13 weeks, total). A

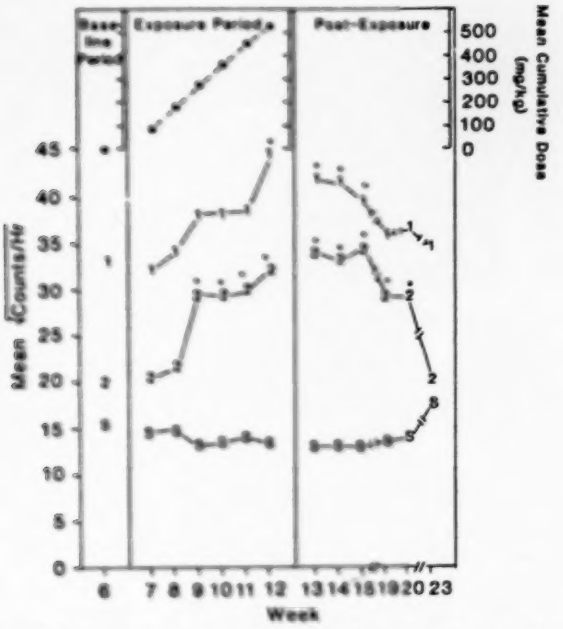

FIG. 3. Locomotor activity for Group ACT before, during and after exposure to 100 ppm acrylamide. (S—S: spontaneous locomotor activity for one hour; 1—1: activity for first hour following d-amphetamine; 2—2: activity for second hour following d-amphetamine; *$p<0.05$ from activity during the baseline-period.

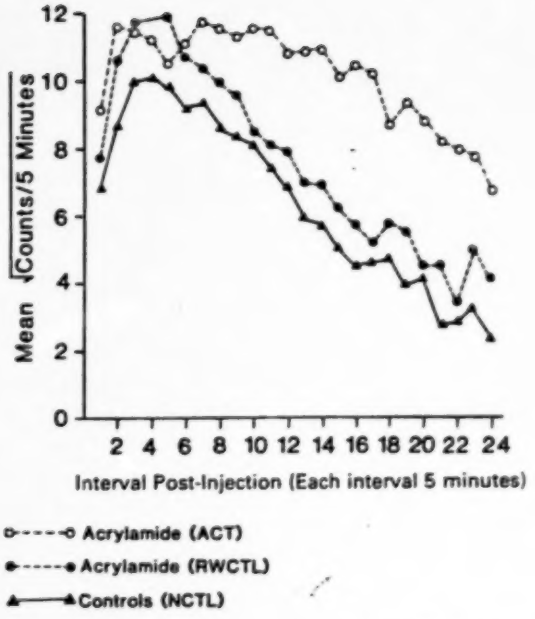

FIG. 4. Locomotor activity for control (NCTL) and previously acrylamide exposed rats (RWCTL) to a single challenge of d-amphetamine during weeks 17–18 of the study. Also included are data from acrylamide exposed animals who were also repeatedly administered d-amphetamine (Group: ACT).

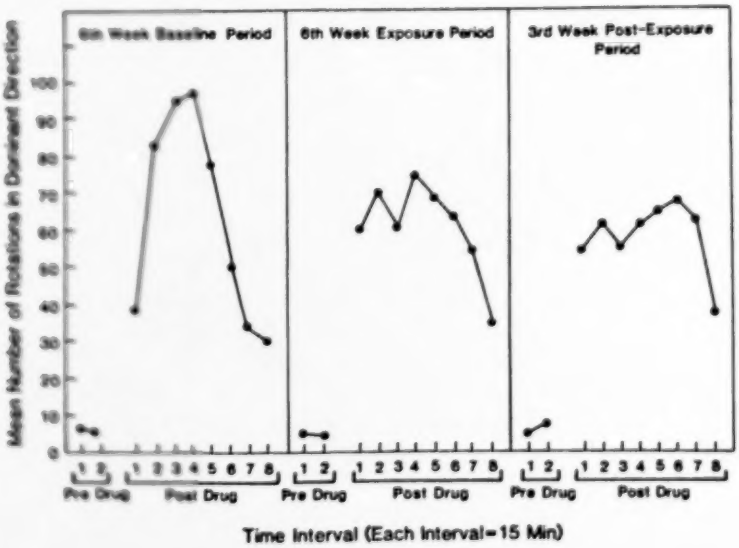
6th Week Baseline Period
6th Week Exposure Period
3rd Week Post-Exposure Period
Mean Number of Rotations in Dominant Direction
Pre Drug
Post Drug
Time Interval (Each Interval=15 Min)

FIG. 5. Spontaneous and drug-elicited (1.0 mg/kg SC d-amphetamine) rotation for three sessions, one prior to exposure, one during the exposure period (cumulative dose ≈547 mg/kg) and one three weeks after termination of exposure.

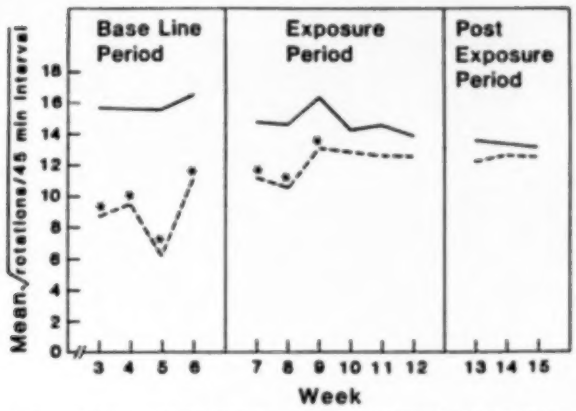

FIG. 6. Rotation 15–45 minutes post-d-amphetamine (solid lines) and 90–120 minutes post-d-amphetamine (broken lines) for Group: ROT (*$p<0.05$).

significant effect for the period-within-a-session, $F(1,6)=17.29$, $p<0.01$, confirmed that the rate of rotation during the earlier portion of a session was greater than the rate of rotation during the later portion. More importantly, this difference in the rate of rotation within a session interacted with the week of testing, $F(12,72)=4.45$, $p<0.001$. This meant that differences between maximum and minimum rotations within a session decreased over weeks of testing. Post-hoc comparisons indicated that differences were evident between the maximum and minimum rates of rotation during each week of the baseline period and during the first 3 weeks following exposure to acrylamide. Throughout the remainder of the exposure period and throughout the period of recovery, there were no statistically significant differences between the rate of turning in the early and late portions of a session.

NCTL and RWCTL groups were tested for drug induced rotation during the 9th and 10th weeks following termination of acrylamide exposure (weeks 21 and 22 of the study). Group RWCTL animals displayed more rotations following drug challenge than did NCTL animals. However, separate two-factor ANOVAs with repeated measures over the eight 15 minute intervals post-injection revealed no significant differences between the groups during either the ninth, $F(1,14)<1$, $p>0.10$, or tenth, $F(1,14)<1$, $p>0.10$, week.

Operant Behavior

The mean number of reinforcements obtained per session is shown in Fig. 7. Although moderately depressed during the second and third weeks of exposure, a repeat measures ANOVA over all weeks of testing failed to show any significant differences in the number of reinforcements obtained across weeks, $F(9,81)=1.60$, $p>0.10$.

Figure 7 also shows the mean percentage of responses emitted during the GO period (%GO=#GO/#GO + #NOGO) for all weeks of testing. Percent data was arcsin transformed prior to a repeat measures ANOVA over weeks of testing. No significant differences were found, $F(9,81)<1.0$, $p>0.10$.

DISCUSSION

Nocturnal activity in running wheels and d-amphetamine induced locomotor activity were the first two behaviors to manifest the effects of chronic exposure to acrylamide. Both behaviors gave initial indications of toxicity during the third week of exposure. The type of changes occurring in these two measures of activity was quite different, however, running wheel activity was decreased from control values while locomotor activity in response to d-amphetamine challenge was augmented above control values.

At least two interpretations can be posited to account for the decrease in activity observed for the running wheel task. One interpretation is that acrylamide could have produced a general malaise which resulted in a decreased motivation to run. A second interpretation is that the decrease in running wheel activity was the result of a gradual deficit in hindlimb function. General malaise seemed an unlikely possibility since there were no differences in the consummatory patterns (food or water intake) between control and acrylamide exposed rats. The latter possibility, that running wheel activity decreased in response to hindlimb neuromuscular dysfunction therefore appeared to be the more plausible explanation. This explanation is consistent with observations of an increased hindlimb splay in a group of rats (Group: RWCTL) for whom running wheel activity was reduced. The simultaneous recovery of running wheel activity and hindlimb splay three weeks after termination of exposure further bolsters this interpretation; although the earlier appearance of altered running wheel activity (by at least two weeks) suggested that this measure was somewhat more sensitive than hindlimb splay in evaluating neuromuscular function.

Spontaneous locomotor activity was unaffected by acrylamide exposure suggesting that this measure was insensitive to deficits in hindlimb function. However, locomotor activity which occurred subsequent to injections of d-amphetamine markedly increased following exposure to acrylamide and only gradually recovered following termination of exposure. There were indications that acrylamide exposed animals repetitively challenged with d-amphetamine-SO_4 (twice/week, 1 mk/kg SC) increased their drug-induced locomotor activity more than acrylamide exposed animals receiving a single 1 mg/kg dose of d-amphetamine. It is not possible to discriminate whether "reverse tolerance" to amphetamine may have interacted with acrylamide exposure since animals comparably challenged with d-amphetamine, but not exposed to acrylamide, were not tested. It can, however, be concluded, based on the locomotor responses of amphetamine naive animals (Groups: RWCTL and NCTL) that acrylamide was independently capable of increasing an animals locomotor response to a single d-amphetamine challenge. This increased sensitivity persisted at least 5–6 weeks beyond the termination of exposure to acrylamide.

It is difficult to ascribe this altered drug response to the occurrence of a peripheral neuropathy. In the rat, aromatic hydroxylation is the primary metabolic pathway for amphetamine [6]. The increased psychomotor activation observed for acrylamide exposed rats could thus have arose from inactivation of this pathway via an effect on hepatic mechanisms [13]. Indeed, in an *in vitro* test [20], addition of acrylamide has been shown to destroy P-450 in the presence of NADH and O_2. Under more realistic conditions, however, *in vivo* administration of acrylamide to rats receiving a total

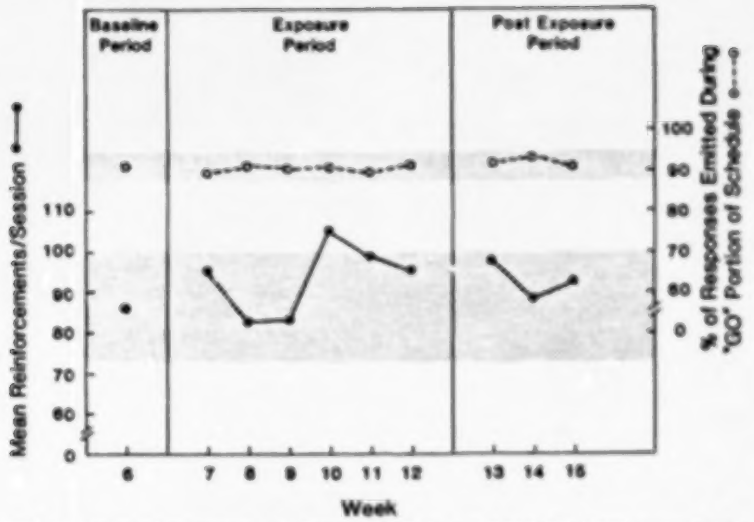

FIG. 7. Repeated assessment of Group OPER in a GO/NO-GO FR20 operant task (shaded area = ±2 S.D. for baseline period).

cumulative dose of 420 mg/kg has been reported to have no disruptive effects on liver UDP-glucuronyl transferase, total hepatic protein, or microsomal protein [14]. It is thus unclear whether the facilitation in amphetamine induced locomotor activity could have been due to hepatic toxicity caused by acrylamide.

Alternatively, exposure to acrylamide may have altered drug-induced locomotor activity via changes in central neurotransmitter function. Animals treated with doses of acrylamide as low as 5 mg/kg/day for 10 days have been reported to show increased ^{3}H-spiroperidol binding to striatal membranes. Modest reductions in striatal membrane protein could not fully account for the increased binding, leading the authors to conclude that dopamine receptors had an increased binding capacity as a result of exposure to acrylamide [2].

This hypothesis is consistent with the results of the current study in which the action of amphetamine on locomotor activity was facilitated.

Rotation in non-lesioned rats is thought to reflect an intrinsic asymmetry in striatal dopamine which is accentuated by d-amphetamine [12]. It is thus of interest that amphetamine-induced rotation was also altered following acrylamide exposure. This change was manifest as a shift from a highly kurtotic distribution over the length of a session prior to acrylamide exposure to a rectangular distribution after several weeks of exposure. Various interpretations may account for this change in responding. However, in view of the locomotor activity data, it appears that animals were rotating at near maximal rates prior to acrylamide exposure and that post-exposure increases in drug responsiveness resulted in overstimulation which interfered with the behavioral response. A subsequent between-group analysis failed to confirm an altered drug response, suggesting that changes in drug responsiveness may have been independent of exposure to acrylamide and solely the result of repeated testing. Alternatively, it is possible that the effects of acrylamide on drug-induced rotation had dissipated by the time between group tests were conducted, ten weeks after exposure to acrylamide had been terminated.

Performance on an operant task was unaffected by exposure to acrylamide. It would be premature to conclude that such findings are indicative of intact "associative" function in acrylamide exposed rats. More demanding operant schedules or changes in stimulus parameters may have increased the sensitivity of the task to acrylamide-related deficits.

In conclusion, the activity of animals in running wheels was affected prior to a "conventional" measure of hindlimb weakness, splaying of the hindlimbs. Operant performance under a FR20/GO-NO-GO schedule was insensitive to the effects of acrylamide; and two measures of amphetamine responsiveness, locomotor activity and rotation, suggested an effect of acrylamide on hepatic metabolism and/or central dopaminergic mechanisms.

REFERENCES

1. Aldous, C. N., R. P. Sharma and C. H. Farr. Acrylamide effects on catecholamine metabolism. *Toxicologist* **1**: 52, 1981.
2. Bondy, S. C., H. A. Tilson and A. K. Agrawal. Neurotransmitter receptors in brain regions of acrylamide-treated rats. II: Effects of extended exposure to acrylamide. *Pharmac. Biochem. Behav.* **14**: 533–537, 1981.
3. Cabe, P. A. and H. A. Tilson. The hindlimb extensor response: A method for assessing motor dysfunction in rats. *Pharmac. Biochem. Behav.* **9**: 133–136, 1978.
4. Cole, S. Brain mechanisms of amphetamine-induced anorexia, locomotion, and stereotypy: A Review. *Neurosci. Biobehav. Rev.* **2**: 89–100, 1978.
5. Dixit, R., R. Husain, P. K. Seth and H. Mukthar. Effect of diethyl maleate on acrylamide induced neuropathy in rats. *Toxicol. Lett.* **6**: 417–421, 1980.
6. Dring, L. G., R. L. Smith and R. T. Williams. The metabolic fate of amphetamine in man and other species. *Biochem. J.* **116**: 425–435, 1979.

7. Edwards, P. M. and V. H. Parker. A simple, sensitive and objective method for early assessment of acrylamide neuropathy in rats. *Toxic. appl. Pharmac.* **40**: 589–591, 1977.
8. Farr, C. H., R. P. Sharma and C. N. Aldous. Acrylamide neurotoxicity: Levels of Tryptophan, serotonin, and 5-hydroxy indoleacetic acid and serotonin turnover in rat brain. *Toxicologist* **1**: 52–53, 1981.
9. Fehling, C., M. Abdulla, A. Brun, M. Dictor, A. Schütz and S. Skerfving. Methyl mercury poisoning in the rat: A combined neurological, chemical and histopathological study. *Toxic. appl. Pharmac.* **33**: 27–37, 1975.
10. Fullerton, P. M. and J. M. Barnes. Peripheral neuropathy in rats produced by acrylamide. *Br. J. ind. Med.* **23**: 210–221, 1966.
11. Glick, S. D., T. P. Jerussi and L. N. Fleisher. Minireview: Turning in circles: The neuropharmacology of rotation. *Life Sci.* **18**: 889–896, 1976.
12. Glick, S. D., T. P. Jerussi and B. Zimmerberg. Behavioral and neuropharmacological correlates of Nigro striatal asymmetry in rats. In: *Lateralization in the Nervous System*, edited by S. Howard, R. W. Doty, L. Goldstein, S. Jaynes and G. Krauthamer. New York: Academic Press, 1977.
13. Goldstein, A., L. Aronow and S. Kalman. *Principles of Drug Action: The Basis of Pharmacology*. New York: John Wiley & Sons, 1974, pp. 246–247.
14. Howland, R. D. and H. E. Lowndes. Hepatic UDP-glucuronyl transferase activity in acrylamide neuropathy. *Experientia* **35**: 248–249, 1979.
15. Jerussi, T. P. and S. D. Glick. Amphetamine-induced rotation in rats without lesions. *Neuropharmacology* **13**: 283–286, 1974.
16. Jerussi, T. P. and S. D. Glick. Apomorphine-induced rotation in normal rats and interaction with unilateral caudate lesions. *Psychopharmacologia* **40**: 329–334, 1975.
17. Jerussi, T. P. and S. D. Glick. Drug-induced rotation in rats without lesions: Behavioral and neurochemical indices of a normal asymmetry in nigro-striatal function. *Psychopharmacology* **47**: 249–260, 1976.
18. Khairy, M. Changes in behavior associated with a nervous system poison (DDT). *Q. Jl exp. Psychol.* **11**: 84–91, 1959.
19. McCollister, D. D., F. Oyen and V. K. Rowe. Toxicology of acrylamide. *Toxic. appl. Pharmac.* **6**: 172–181, 1964.
20. Patel, J. M., E. Ortiz and K. C. Leibman. Destruction of hepatic cytochrome P-450 by allylic industrial toxicants. Federation Proceedings, 65th Annual Meeting, Atlanta, Georgia, 1981. (Abstract #2325)
21. Prineas, J. The pathogenesis of dying-back polyneuropathies. Part II: An ultrastructural study of experimental acrylamide intoxication in the cat. *J. Neuropath. exp. Neurol.* **28**: 598–621, 1969.
22. Segal, D. S. and A. J. Mandell. Long-term administration of d-amphetamine: progressive augmentation of motor activity and stereotypy. *Pharmac. Biochem. Behav.* **2**: 249–255, 1974.
23. Spencer, P. S. and H. H. Schaumburg. A review of acrylamide neurotoxicity. Part II. Experimental animal neurotoxicity and pathologic mechanisms. *Can. J. neurol. Sci.* **1**: 152–169, 1974.
24. Spencer, P. S. and H. H. Schaumburg. Ultra-structural studies of the dying-back process. IV. Differential vulnerability of PNS and CNS fibers in experimental central-peripheral distal axonopathies. *J. Neuropath. exp. Neurol.* **36**: 300–320, 1977.
25. Winer, B. J. *Statistical Principles in Experimental Design*. 2nd edition. New York: McGraw-Hill, 1971, pp. 397–402.